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14. ABSTRACT

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Keywords: *Dehalococcoides*; reductive dechlorination; dehalorespiration, PCE; TCE; DCE; chlorophenol; microarrays; transcriptional regulation; transcriptome; continuous feed cultures, batch fed cultures, pseudo-steady state transcript levels, and reductive dehalogenases.

Abstract:

Bacteria of the group "*Dehalococcoides*" display the ability to respire recalcitrant chlorinated organic compounds. "*Dehalococcoides*" strains' respiratory pathways and function of most genome-encoded enzymes responsible for dechlorination, reductive dehalogenases (RDases), remain incompletely annotated. To further the description of the biological organization of "*Dehalococcoides*", this study monitored the trancriptomic response of "*Dehalococcoides ethenogenes*" stain 195 using two-color microarrays. This study analyzed the transcriptome of 23 varied continuous feed (or pseudo-steady state (PSS)) conditions and two distinct batch fed conditions. The continuous feed experiments were comprised of 57 cultures with varying electron acceptor feed rates (0-504 μeeq/(L-hr)), electron acceptor types (tetrachloroethene (PCE), trichloroethene (TCE), dichloroethene (DCE), 2,3-dichlorophenol (DCP), and no electron acceptor), electron donor to acceptor ratios (0.7 to 17 on an electron equivalence (eeq) basis), and electron donor type (butyrate, lactate, yeast extract, fermented yeast, pure hydrogen, or endogenous biomass decay). When similarly respiring (~120 μeeq PCE/(L-hr)) batch and PSS cultures were contrasted, the RDases DET1545 and DET0180 were up-regulated in the PSS cultures indicating activity at lower overall electron acceptor concentration. For all continuous-fed chloroethene cultures, members of the RDase family and electron transport chain displayed unique clusters of transcripts responding either positively, negatively, or indifferently to the respiration rate. An RDase within the indifferent group, DET1171, was highly (31 ± 15fold) up-regulated in response to DCP being fed as the electron acceptor. DET1171 could potentially play a role with DET1559 (27±4.1 fold up-regulated during DCP growth) and DET0318 *pceA* in the dechlorination of chlorophenols.

Introduction:

Recalcitrant halogenated organics contaminate a majority of active hazardous waste cleanup sites (55). Site managers and engineers have turned toward utilizing populations of the bacteria "*Dehalococcoides"* (DHC) in bioremediation efforts (47). DHC strains are capable of anaerobicallyreductively dehalogenating halogenated compounds including chlorinated-ethenes (48,50,54), -ethanes (18,25,54), -phenols (2,21), -benzenes (1,3,21), -dioxins (11,13,22), -dibenzodioxins (21), -dibenzofurans (46), -naphthalene (21), and -biphenyls (8,21,80) allowing for flexibility in the bioremediation application of this bacteria. Additionally, DHC is often found and active at chlorinated organic contaminated sites (7,8,10,12,14,24,31,35,44,81-82) and need only to be biostimulated to begin remediating the chlorinated organic compounds. Several case studies focusing on bioaugmentation of DHC at PCE and/or TCE contaminated sites have also showed success (19,45,52,67).

The first strain of DHC identified, "*Dehalococcoides ethenogenes* strain 195" (DET), was isolated in 1997 (53) and its genome sequenced, assembled, and annotated in 2005 (69). Genomes of additional DHC members, such as CBDB1 (42,74), VS (58,75,49), GT (71), and BAV1 (27,40,49), have been sequenced and annotated since, with other members being isolated and/or characterized (e.g. strain FL2(28), KB1(17) metagenome available through the Joint Genome Institute). However, much of the biological organization of DHC remains unknown. DHC is an obligate hydrogenotroph and dehalorespirer (32). The identified (69) putative enzymes and regulatory elements comprising the electron transport chain include a unique ensemble of reductive dehalogenases (RDases), which reduce halogenated organics; hydrogenases, which oxidize DHC's sole electron donor (hydrogen); and other oxidoreductases of unknown or misannotated function (e.g. Fdh, format dehydrogenase; Nuo, NADH ubiquinone oxidoreductase; Mod, molybdopterin containing oxidoreductase). Every DHC strain has an unique subset of over 90 RDases (41) likely imparting capabilities for dehalogenating different chlorinated substrates in unique patterns (33,34,51,79). Of DET's seventeen functional RDases, DET0079 (TceA),

DET0318 (PceA), DET1559, and DET1545 are the most investigated across multiple experiments within a mixed community culture containing DET as the sole dehalogenator (Donna II) (23,36,37,43,51,60-62,75). DET's NiFe hydrogenase Hup is the only hydrogenase predicted to face the periplasm (56,69,73). Although RDases, Hup, and other membrane bound oxidoreductase proteins have been documented in several DHC studies (57,77), the complete organization and interaction between these respiration elements is not yet fully understood (59). Observations of broad expression patterns across many experiments generate hypothesizes about which transcripts are working in concert to fulfill specific biological functions.

To broaden the understanding of the biological organization of "*Dehalococcoides*", this study employed microarray technology to monitor the transcriptomic response of "*Dehalococcoides ethenogenes* stain 195" in a mixed community culture to 19 varied experimental continuous-feed or pseudo steady state (PSS) conditions (a total of 57 total cultures are monitored considering biological replicates). In these studies, PSS conditions refer to a steady mRNA transcript level and not biomass. Previous transcriptomic studies performed on DHC include characterizing unknown species or cultures (16,78) and monitoring both pure and mixed well defined cultures (37,76). The microarray studies investigating the entire transcriptome under pure-culture and batch fed conditions with trichloroethene (TCE) and hydrogen fed (as the electron acceptor and electron donor respectively) (37) revealed key insights into the organism's time-dependent response, carbon metabolism (72), corrinoid response (38), and predicted nitrogen fixation pathway (43).

In order to extend the existing microarray datasets and more closely simulate field conditions this study analyzed the transcriptome of cultures grown in conditions with varying electron acceptor feed rates (from 0-504 μeeq/(L-hr)), electron acceptor types (between tetrachloroethene (PCE), trichloroethene (TCE), dichloroethene (DCE), 2,3-dichlorophenol (DCP), and no electron acceptor), electron donor to acceptor ratios (from 0.7 to 17 on an electron equivalence basis), and electron donor type (between butyrate, lactate, yeast extract, fermented yeast, pure hydrogen, or

endogenous biomass decay only). By continuously feeding substrates over the course of days, a near constant (PSS) pool of mRNA was established. A large enough dataset potentially provides the association of specific genes' perturbations to gradations in experimental conditions rather than responses confounded by non-steady conditions or batch growth. By monitoring the transcriptome, the genome-wide response can be described. Additionally, the growth of DET in a non-pure culture setting allows a fuller insight into the maintained mixed community cultures (Cornell Donna II (53), Pinellas (26), Victoria (30), KB-1 (18) and ANAS cultures (64)) and a better representation of field conditions in which DET will compete with methanogens for hydrogen produced from organisms that ferment organic substrates, when provided (20,65,70).

Materials and Methods:

Culture Growth Conditions

All subcultures studied in this experiment were 100 mL samples from a maintained 6L mixed community culture (Donna II) previously described (20,60-62,65) The culture contains a DET population (near 5×10^8 cells/mL; 60% of total population on per cell basis) mixed with methanogens and organisms that ferment organic substrates.

Continuous Feed or Pseudo-Steady State Setup (PSS):

The continuous feed or pseudo-steady state (PSS) culture setup has been previously described (60). In brief, 160 mL glass serum bottles were filled with 100 mL of 3-day starved mixed community culture sampled from the main reactor (Donna II). Varying combination of Pressure Lok syringe volumes (10 mL, 5 mL, 2.5 mL, 500 μL, and 100 μL; VICI Precision Sampling) and concentrations of electron acceptor and donor in media were loaded on a Cole Palmer 74900 Syringe Pump to deliver an expected continuous rate.

Gas Chromatography Monitoring

Chlorinated-ethenes, ethene, and methane were monitored utilizing a gas chromatography method previously described (62). Chlorinated phenols were monitored utilizing a modified method previously outlined (39). The chlorinated phenol samples were comprised of 1 mL liquid samples

withdrawn from the subcultures and frozen. The thawed samples were transferred to a 1 mL glass vial, 100 uL of hexane was added to the sample, and the vial was shaken at 300 rpm for six hours. The FID gas chromatography method selected has an injection temperature of 200° C, a constant column temperature of 160° C, and the FID detector temperature of 200° C on a Supelcowax 10 column (30 m × 0.53 mm with 1.00 μm film, Supelco). The gas mixture was 200 kPa nitrogen as the carrying gas, 200 kPa air, and 250 kPa hydrogen. 5 μL of the sample in hexane was injected. Chemical standard curves were made in both hexane and media from pure chlorinated phenols (ACROS Organics).

Calculation of respiration rates

Respiration is considered to occur for the higher chlorinated ethenes to VC or ethene (60) with 6, 4, and 2 electron equivalents (eeqs) for PCE, TCE, and DCE respectively with the specific calculation previously published (61). Extending this analysis to the dichlorophenol DCP experiments, monochlorophenol (MCP) is the terminal product (21). Therefore, DET will receive 2 eeqs per mol DCP. The respiration rate equation for DCP, r_{DCP}, in μeeq/L/hr is:

$$r_{DCP} = 2\frac{\mathrm{d}(MCP)}{\mathrm{d}t}$$

Microarray Design:

The microarray designed for this experiment was an Agilent Technologies© two-color, 15k, 60 mer, 8 plex array. The specific designs of the probes utilized a modified method provided by the eArray © software suite (5). The first probe set utilized for the batch versus PSS experiment employed a base-composition (BC) technique for designing and scoring the best probe for each transcript (5). The second probe set redesigned for the remainder of the experiments was expanded to include community members, non-protein encoding RNA transcripts (rRNAs, tRNAs), and a luciferase control. A modified temperature matching (Tm) method developed the probe set for redesigned array. The Tm method searched for an optimal design with a consistent melting point temperature (80° C) while sacrificing the overall quality of the individual probe (5).

However, if a BC score of 3,4, or poor was reported for a transcript, multiple probes around the melting temperature of 80° C were designed. The probe with the best base composition score nearest to the 80° C temperature was selected. The designs were searched using the Basic Local Alignment Search Tool (BLAST) (15) against both the National Center for Biotechnology Information (NCBI) nucleotide collection and the assembled mixed community metagenome (metagenome data not reported, currently being compiled by the Joint Genome Institute) to confirm the specificity of all probe sets. Both microarray platform designs are uploaded and freely available at the NCBI Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/).

RNA-cDNA Handling for Microarray Monitoring

50 mL of liquid culture samples were centrifuged-at 14190×g. The centrifuged sample was split into 8 individual RNA extractions with each sample following the RNeasy© Mini Kit (Qiagen) extraction previously outlined (62). The 8 distinct RNA extractions were recombined on the spin filter before the first RW1 buffer wash. The Superscript I © DNAse RNA cleanup, amino-allyl cDNA formation, cDNA cleanup, and cDNA labeling with Cy3 or Cy5 followed the method outlined (76). The quality and quantity of the RNA was determined using the RNA 6000 Nano assay on an Agilent 2100 bioanalyzer (Agilent Technologies). The quantity of resulting cDNA was determined by using the Quant-IT™ OliGreen® ssDNA Assay Kit (Invitrogen). A common control RNA pool sampled from the main Donna II reactor after 3 days of starvation was labeled with Cy3.

Microarray Hybridization and Scanning:

For each experiment, Cy5 labeled cDNA from the mixed community mRNA pool was hybridized against an aliquot of common control of Cy3 labeled cDNA from 3-day starved culture. The hybridization, washing, and scanning of the microarray samples was performed by the Cornell University Microarray Core Facility (http://cores.lifesciences.cornell.edu/brcinfo/) and followed the methods outlined by the manufacturer (5). The general procedure mixed 25 µl (~400 ng) of

the labeled cDNA sample with 25 µl 2x Gene Expression (GEx) Hybridization Buffer HI-RPM (5), hybridized the sample to the microarray slide at 65° C for 17 hours, washed with GEx Wash Buffer 1 and 2 (5) at room and elevated (37° C) temperatures, and scanned with an Agilent Technologies Scanner G2505C with a 5 µm resolution.

Statistical Treatment of the Data Set:

Microarray image analysis was conducted using Agilent Feature Extraction 10.5 Image Analysis Software. The Feature Extraction Software was also utilized to perform a within array modified LOESS normalization between the Cy5 and Cy3 signals, to calculate a log ratio between the Cy5 and Cy3 channels, and to calculate a modified Student t-test p-value between the Cy5 and Cy3 signal distributions (83). A between array normalization was not performed due to the wide variety of experimental conditions analyzed, possibly violating the assumption that less than 20% of transcripts are differentially regulated throughout the experimental set. The more detailed treatment the Agilent Feature Extraction employed can be found in the user manual (4). Replicate spots for the same probe (ranging from 6-20 spots/probe) were geometrically averaged. Hierarchical clustering was performed in R 2.11.1 (http://www.r-project.org/). The K-Means cluster, figure of merit (FOM) test, and the Cluster Affinity Search Technique were performed in the Multiple Experiment Viewer (MeV 4.6) freely available from JCVI (66). The raw and normalized data is freely available at the NCBI GEO database.

Results:

Batch Growth Compared to the Continuous Feed Pseudo Steady State System

The first set of microarrays compared the mixed community culture with similar electron acceptor/donor conditions under batch and pseudo-steady state growth. The batch culture dechlorinated 660 µeeqs/L of PCE to VC or ethene in 6 hours (110 µeeqs/(L-hr)) while the PSS experiments received a continuous input of 53±13 or 83±2.8 µeeqs/L-hr of PCE or TCE respectively. The main biological differences are the high initial pulse of electron acceptor and donor seen by the batch cultures to the low concentrations maintained in the PSS-setup (the batch

culture experiences the initial pulse of 660 μeeqs/L while the PSS cultures maintain a constant level of about 12±9.5 μeeqs/L of chlorinated ethenes based on the last 48 hours of GC data) and that the rate of continuous feeding in the PSS cultures was maintained for 7 days. The two PCE (1 & 2) and two TCE (1 & 2) experiments are compared via a correlogram (Figure 1). The PCE (1 & 2) cultures do not display a high correlation with each other due to the inadvertent variation in the feeding of electron acceptor (PCE 1and 2 were fed 66 and 44 μeeq/(L-hr) respectively, (GC data displayed in Supplemental Image 1). To determine the transcripts differentially regulated similarly across all four arrays, only transcripts where all replicate probe spots (n=6-20) on the microarray exceed a fold ratio between the PSS and batch cultures of two (for up-regulated genes) or one-half (for down-regulated genes) and are below a p-value of 0.05 are considered. Overall, compared to the batch control, PCE 1, PCE 2, TCE 1, and TCE 2 displayed 193, 165, 144, and 275 differentially regulated transcripts, respectively. Of these, 43 (23 up- and 20 down-regulated) are shared across all samples and displayed in Table 1. Within the 23 up-regulated transcripts, the RDase DET0180 and its anchoring protein as well as the RDase anchoring protein DET1544 display a 5.5±4.11, 5.1±0.92, and 3.5±0.59 fold increase respectively. The presence of the anchoring protein DET1544 without the catalytic RDase DET1545 transcript is surprising as they are predicted to reside on the same polycistronic mRNA. All probes for the RDase DET1545 pass the requirement of displaying a p-value below 0.05, but the transcript is excluded from the stringent analysis due to two probe spots (out of 20 replicates) in the PCE1 experiment failing to exceed the ratio cut off of 2. If the stringency of the analysis is relaxed to remove the log ratio cutoff, the number of transcripts shared across all experiments is increased from 43 to 65 (Supplemental Table 1). DET1545 falls within this category displaying an average ratio across all experiments of 3.3±0.86. The 20 significantly down-regulated transcripts under PSS conditions in the stringent analysis are populated by many key transcripts encoding for enzymes in respiratory energy conservation. These include transcripts of the *ATPase* operon (DET0558, DET0560, DET0561, DET0562) and subunits of the hydrogenase *ech* (DET0864). Additional members of

the *ech* operon (DET0861 and DET0862) are found in the expanded data set (Supplemental Table 1).

Expression profiles comparison across multiple PSS experiments:

In order to explore a wide variety of environmental conditions, mRNA pools from 53 experiments (performed on 14 separate days) were analyzed by the microarray technique described above. The parameters and conditions for each experiment are outlined in Table 2. The electron acceptors were varied from none, PCE, TCE, DCE, and DCP while the electron donor for the PCE experiments was varied from none, butyrate (with yeast extract (YE)), lactate (with YE), hydrogen, YE, and fermented yeast extract (FYE). The feed rates of electron acceptor and the ratio of electron acceptor to donor were altered as well. The respiration rates varied between 0 μeeq/(L-hr) in cultures not fed electron acceptor to 170 μeeq/(L-hr) in the PCE High Experiments.

When the experiments are organized via a hierarchal Ward cluster dendogram based on a Euclidean distance matrix of the log ratio fold changes (Figure 2), two distinct groupings develop. The two clusters are separated primarily by the respiration rate recorded with higher and lower respiration rates clustering together. However, the High 2 from the High-Low PCE experiment (HLH2_INHIB) plots with the higher respiration cluster when in fact respiration ceased prior to culture sampling due to the formation of non-aqueous phase PCE. HLH1_INHIB and HLH2_INHIB display a unique decay series as both cultures experienced an initial high respiration rate (~100 μeeq/(L-hr)), but PCE built up above its solubility limit at day 2 inhibiting PCE respiration. Additionally, the DCE "3 rate" mid-range (DCE B1 and B2) do not fall into the same cluster. The remainder of the experiments and biological replicates plot as expected. The hydrogen abiotic controls (H2A1 and A2) cluster with the 3 day decay experiment (DecayA1) as both are decay experiments on the same time scale. Additionally, the experiments fed: PCE with either no electron donor, YE, or FYE with PCE (P0FY_XX); the chlorophenol experiment (Cp);

the PCE low series (PLL); the decay 7-day (DecayB) replicates; the PCE high series (HiP); the half butyrate series (PHB); and the majority of the "3 rate" experiments (P3, T3, and D3) cluster with their respective conditions and biological replicates, supporting the biological reproducibility of the overall transcriptional response.

Gene clusters

After examining the clustering of experiments, the clustering of genes with similar behavior was explored with a K-means clustering technique. This technique was able to align all genes into biologically relevant clusters (63). However, the hindrance to employing a K-means clustering technique was that the number of clusters assigned must be set prior to analysis. Based on a figure of merit test, the number of biological clusters was set at 20. A hierarchal dendrogram displaying the relationship among the twenty clusters is displayed in Figure 3 with the specific gene list of each cluster described in Supplemental Table 2. Other clustering techniques, such as the cluster affinity search technique (CAST) (9), gave similar results (data not shown). The 20 clusters map into four distinct superclusters. Figure 3.b-e displays the cluster's centroid log ratio expression value versus respiration rate broken into the four primary clusters. Figure 3.b displays a general positive trend in response to respiration rate while Figure 3.c displays a general negative trend (Figure 3.d-e displays clusters with centroids of these clusters remain nearly constant). This bifurcation is important to note as the majority of high respiration rate linked RDases (*tceA*, *pceA*, DET1559) and respiration chain elements (*hup*, *ATPase*) are members of the clusters plotted in Figure 3.b while the low respiration linked RDases (DET0173, DET1535, DET1538, and DET1545) are members of the clusters plotted in Figure 3.c.

Across all clusters, three centroid data points occurring around the 35 μeeq/(L-hr) respiration rate appear to be outliers (lower in Figure 3.b and higher in Figure 3.c). These centroids are noteworthy as they represent the DCE_INHIB experiments. If the final 48 hours of respiration prior to sampling are considered, the respiration rate was actually around 2.4±1.8 μeeq/(L-hr)

which shifts all three centroids to the left in the respiration diagrams of Figure 3.b-e. Following this adjustment, these points do not appear to be outliers but support the overall trend.

K-Means Clusters Mapped onto DET's Genome:

-Describe binning the genes based on supercluster

-Show that there are several regions where a siginificant number of genes appear in the same supercluster

-Describe the virus region and explore other genes that are clustered with it.

Average Expression Values Across all Experiments

-Describes those genes that are most highly expressed

-Compare to Johnson et. al to see if any uniquely turned on

Putative Respiration Chain Element Rate Response

The fact respiration is a key and informative phenotype of DET prompted interest in plotting expression patterns of transcripts versus experimental respiration rate. Focusing on the responses of mRNAs encoding for known or predicted respiration chain elements could determine if there are unique electron transport chain members responding to low or high respiration. Figure 4 displays the expression pattern clustering of 18 RDases (17 full length plus DET0162, an RDase with a known point mutation (69)), 5 hydrogenases (*hym*, *hyc*, *ech*, *hup*, *vhu*), other membrane-bound oxidoreductases (*mod* (potential molybdopterin oxidoreductases), *fdh* (annotated formate dehydrogenase), *nuo* (NADH-ubiquinone-oxidoreductase)), and *ATPase*. In this figure a single polypeptide subunit is selected to represent the full operon. However, members of the electron transport chain often are encoded on multi-subunit operons. A version of Figure 4 with all subunits plotted is presented in Supplemental Figure 2. The majority of subunits of the respective operons cluster closely together. However, variability is seen in the *nuo*, *ech*, and *hym* transcripts. In Figure 4, three groups emerge and again vary in their trend relative to respiration rate. The first grouping contains the high-respiration-linked RDases with the majority of the other predicted respiratory elements. As seen by Figure 4.b, these transcripts respond very sharply to increased

levels of respiration and then hit a plateau. This plateau was noted in previously published work by Rahm and Richardson (60,61). The low-respiration-linked RDases, DET0173, DET1535, DET1538, and DET1545, are tightly clustered in the second group and at rates above 5 μeeq/(L-hr) display an inverse relationship to respiration as first noted in the K-means analysis (Cluster 1 in Figure 3.c). The remaining RDases and the putative respiration chain elements *mod* and *ech* appear to fall within a cluster that does not respond greatly to varied levels of chlorinated ethene respiration although clustering between *modA* (DET0103) and DET0180 is observed (Figure 4.a).

Response of Respiration Elements to Switching the Electron Acceptor Type from Chlorinated Ethenes to 2,3-Dichlorophenol and 1,2,3,4 Tetrachlorobenzene

The electron acceptor was varied in the experimental set among PCE, TCE, DCE or 2,3-dichlorophenol (DCP). Figure 5.a displays the natural value differential expression of the individual RDases across a relatively consistent low-rate of respiration with varying the electron acceptor type (electron donor was always butyrate). The *tceA* transcript is about equally up-regulated in the DCP samples as with any of the chlorinated ethenes (PCE, TCE, DCE) (Figure 4.a). Additionally, DET1171 and DET1559 are both highly up-regulated in the chlorophenols fed cultures (32 ± 16 and 27 ± 4.1 fold respectively). The distribution of the absolute intensity of RDase spots for the varied electron acceptor types (Figure 5.b) reveals that for the TCE and DCE fed cultures *tceA* dominates the percentage of the RDase transcriptional pool, for the PCE fed culture DET0162 dominates, while for the DCP fed culture *pceA* is the dominant transcript comprising above 40% of the total RDase processed fluorescence. Additionally, *pceA*'s differential expression displays a slight non-significant up-regulation for the DCP culture as compared to the chlorinated ethene fed cultures. Furthermore, DET0180 sees a decrease in the percentage of RDase intensity between DCP and TCE/DCE.

Including the other respiratory chain elements in the analysis (data not shown) reveals that the majority of the transcripts are down regulated under DCP conditions. However, *nuo* (DET1571),

vhu (DET0615), and *ech* (DET0860) do not display a significant difference between the DCP fed and chlorinated ethene fed cultures in terms of ratio to standard controls.

The analysis for DCP specific response was expanded to include all transcripts in a rank ordered list based off of differential expression ratios for the DCP experimental series (Supplemental Table 3). The first entry is DET1171, as previously indicated in Figure 5.a. Besides the two RDases DET1171 and DET1559 reside in the top twenty of all genes by fold change, response regulators and hypothetical proteins heavily populate the list. Two histidine kinase sensory boxes (DET0301 and DET1560), *pyrR* (DET1198, putative transcriptional binding protein of pyrimidine nucleotide biosynthesis), and *recX* (DET1607, putative regulatory protein for the DNA recombinase RecA) all display an up regulation greater than ten-fold. Among the most down-regulated transcripts, an over abundance of genes related to iron or cobalamin are present. These genes include two ABC transporters (DET0650 (corrinoid specific) and DET1176) and a biotin-Acetyl-CoA-carboxylase ligase (DET0849, also putatively described as a transcriptional repressor).

Tetrachlorobenzene experiments (two weeks)

Tetrachlorobenzene arrays (one week)

Analysis (few days)

Discussion:

In the comparison of the batch fed culture to the PSS setup, the RDases DET1545 and DET0180 are displayed to be highly up-regulated under PSS conditions. This suggests that DET1545 and DET0180 are up-regulated in response to lower electron acceptor availability conditions (specifically the batch fed culture processed a 660 μeeq/(L) pulse of PCE at a rate of 110 μeeq/(L-hr) while the PSS received a continuous low rate of 55±8.2 μeeq/(L-hr)), as supported by previous publications (60-62). Although neither transcript is dominant in the absolute intensity sense (data not shown), the minor RDases potentially serve as key enzymes when electron acceptor availability is low or, their regulatory elements are sensitive to external electron acceptor

concentrations. When the analysis is expanded to include putative members of the entire respiration chain, it is noted that the *ech* and *ATPase* operons are both down-regulated. The ATPase enzyme encodes for a proton pump utilized primarily for energy generation and the Ech hydrogenase putatively encodes for a proton pump which generates low potential electrons that could be used to reduce ferredoxin or possibly cobalt in RDase corrinoids to the Co I state, or for other biosynthetic metabolites (69). Potentially, the batch cultures are limited by the rate of hydrogen production from butyrate while the continuous-fed cultures are limited for electron acceptor therefore allowing higher hydrogen levels. The higher hydrogen level in the continuous feed cultures may mitigate "*Dehalococcoides*" need for reverse electron transport by Ech.

In previous work with the mixed community culture Donna II, qRTPCR was used to monitor expression levels of selected genes (60-62). The trends from that work with respect to the RDases' relationship to respiration rate are supported here with genome wide profiles. Namely, some highly expressed respiration chain elements and RDases up regulate sharply in response to increased respiration rate or electron acceptor concentration before plateauing at the highest rates while, in contrast, other RDases are up-regulated only at low respiration rates or electron acceptor concentrations before being down-regulated at higher rates. This trend is also supported by batch studies with DET where the low-respiration rate linked cluster of RDases is up-regulated upon entry into stationary phase (37). This bifurcation of behavior in the RDases may indicate a unique response of specializing RDases in the electron transport chain and prove useful for the identification of field site biomarkers in determining the respiration state of the organisms in situ. However, though the expression patterns of these RDases are remarkably repeatable, their substrate range remains unknown and functional studies are needed.

Focusing on responses specific to electron acceptor type, previous studies comparing PCE, TCE, and DCE at similar respiration rates showed shifts in relative expression of the RDases being tracked by qRTPCR (e.g. *pceA* was down-regulated slightly when TCE or DCE was fed), but these shifts were less than two fold (61). Shifting to a different class of chloroorganic electron

acceptor altogether displayed larger shifts (2,21,23). The current study, in varying the electron acceptor type from chlorinated ethenes to DCP, supports the majority of reported findings (23). DET1559 and *pceA* have been highlighted as two transcripts potentially involved in chlorophenol dechlorination (23). These two findings are supported in the current study by the dominance of *pceA* in the RDase absolute intensity distribution (Figure 5.b), and the high up-regulation of DET1559 (27 ± 4.1 fold for DCP compared to 4.4 ± 1.4 fold for PCE). PceA has been shown to be bifunctional, able to dechlorinate both chlorinated ethenes and phenols (2).

Expanding off of these findings, the current study displays that the RDase DET1171 is highly up regulated in the DCP cultures (31 ± 15 fold) while not significantly up-regulated in the chlorinated ethene fed cultures (Figure 5.a). In the Fung et al. 2007 study, the RDase DET1171 is indicated as, but not highlighted for due to low overall expression, displaying significant up regulation. When DET1171's amino-acid sequence was searched by BLAST against the NCBI protein database, ortholog RDases appear in the CBDB1, BAV1, and VS strain (95%, 95%, and 97% amino acid identity respectively) as previously indicated (49). Additionally, weaker hits match with the ortho-chlorophenol RDases in the *Desulfitobacterium* genus (39% amino acid identity) with seven out of the top twenty hits described as a chlorophenols-related RDase. However, the closest hits outside of the "*Dehalococcoides*" group are genes from species of *Photobacterium* and *Shewanella*, which may be non-energy conserving RDases involved in the breakdown of a chlororganics. Therefore, the correlation of DET1171 with DCP dechlorination may be the result of shared transcriptional regulation rather than actual functional dechlorination by the final enzyme. To fully explore the function and behavior of DET1171 and the other RDase family members, the dataset which is primarily comprised of chlorinated ethene fed cultures should be expanded to explore more perturbations in chlorophenols, chlorobenzene, chlorodibenzodioxins, and other chlorinated substances.

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Figure 1. Comparison of the microarray results for the Batch and PSS fed cultures. The correlogram displays the similarity of experimental replicates TCE 1&2 and PCE 1 & 2 by correlating the gene expression across experiments for all transcripts compared to batch conditions.

Table 1. The 43 genes that are similarly regulated amongst all four Batch vs. PSS samples with the transcript name, regulation (up or down when comparing PSS to batch level), average natural value ratio recorded, and the standard deviation of that ratio across all 4 arrays (40 spots on 4 arrays).

Table 2. Experimental conditions for the continuous-feed (PSS) investigations. The experimental title provides a brief description of the overall experiment where the culture title designates the electron acceptor (PCE, TCE, DCE, DCP, or none (-)) and electron donor source (butyrate, lactate, hydrogen, yeast extract (YE), fermented yeast extract (FYE), hydrogen, or none (-)) type. Note, the experiments that received butyrate and lactate also were received YE. Additionally, the

Batch versus PSS direct arrays are not displayed as those experiments were compared against a different control.

Figure 2. Full experimental Ward cluster dendogram for all experiments based off of an Euclidean distance matrix for the log ratio fold change of all gene targets. Consult Table 2 for culture descriptions.

Figure 3. Breakdown of the of individual genes' expression patterns into clusters. (a) A Euclidean distance and Ward cluster dendogram comparing the similarities of the clusters. (b-e) Scatterplots plotting the centroid value (with 95% confidence intervals about the mean) against the experimental respiration rates for all chlorinated ethenes.

Figure 4. Respiratory chain elements' response to respiration rate. (a) Ward hierarcial clustering with an Euclidean distance matrix of the differential expression in log ratio. Transcripts with an (*) do not exceed the intensity cutoff limit of 100. The bootstrap analysis of the cluster results in two scores, the approximately unbiased (AU) p-value in red and the bootstrap probability (BP) in green. (b-d) Scatterplots of the differential expression log ratio for individual genes in each large cluster plotted against the respiration rate (μeeq/L/hr). The black point represents the average of the log ratio values in each gene cluster with the error bars representing the 95% confidence interval about the mean.

Figure 5. RDase distribution across different electron acceptors for the PCE, TCE, DCE, DCP, and No Acceptor fed conditions (a) natural value ratio compared to a 3-day starved culture for the 17 RDase and the *rpoB* transcript is plotted. Error bars represent standard error across biological replicates. (b) The absolute processed intensity value distribution of the RDases.

Table 3. Correlation scores of RDases that meet the background cutoff to neighboring annotated genes including putative response regulators. The solid black line separates the RDases based on their clustering and response to respiration rate (RDases responding positively to respiration rate above the black line, negatively below) displayed in Figure 4. A negative correlation score indicates an inverse correlation. Values >0.25 or <-.25 are considered significant by the one-tailed t-test with N=49.

Table 4. Correlation scores of *tceA* to RDases and response regulators adjacent to RDases (left column) and correlation scores of *tceA* to all transcripts on DET's genome represented on the microarray (right column).

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	PCE1	PCE2	TCE1	TCE2
PCE1	1	0.72	0.78	0.78
PCE2	0.72	1	0.66	0.55
TCE1	0.78	0.66	1	0.84
TCE2	0.78	0.55	0.84	1

Figure 1. Comparison of the Batch and PSS fed cultures. The correlogram displays the similarity of experimental replicates TCE 1&2 and PCE 1 & 2 by correlating the gene expression across experiments for all transcripts.

Transcript ID and Description	Regulation	Ratio	StDev
DET0036 hypothetical protein	DOWN	0.44	0.04
DET0137 mgsA methylglyoxal synthase	DOWN	0.31	0.11
DET0180 reductive dehalogenase	UP	5.5	4.11
DET0181 reductive dehalogenase anchoring protein	UP	5.1	0.92
DET0297 hypothetical protein	UP	4.9	3.03
DET0368 proS prolyl tRNA synthetase	UP	2.7	0.25
DET0423 hypothetical protein	UP	4.2	5.14
DET0558 atpB ATP synthase F0, A subunit	DOWN	0.21	0.04
DET0560 atpF ATP synthase F0, B subunit	DOWN	0.33	0.08
DET0561 atpH ATP synthase F1, delta subunit	DOWN	0.33	0.05
DET0562 atpA ATP synthase subunit A	DOWN	0.41	0.04
DET0673 hypothetical protein	UP	3.6	1.21
DET0721 hypothetical protein	DOWN	0.36	0.04
DET0736 oxidoreductase	UP	2.7	0.42
DET0754 hypothetical protein	UP	2.7	1.12
DET0755 hypothetical protein	UP	3.8	0.58
DET0864 hydrogenase subunit HymB, putative	DOWN	0.37	0.09
DET0908 arsenical pump membrane protein, putative	UP	7.8	2.82
DET0909 hypothetical protein	UP	2.8	0.35
DET0945 ABC transporter	DOWN	0.43	0.05
DET1021 hypothetical protein	DOWN	0.20	0.08
DET1035 tryptophan synthase subunit beta	UP	3.2	0.40
DET1038 ileS isoleucyl tRNA synthetase	UP	2.5	0.39
DET1051 hypothetical protein	UP	3.8	0.61
DET1063 DNA binding response regulator, LuxR	UP	2.7	0.81
DET1092 DNA methylase	DOWN	0.41	0.07
DET1106 hypothetical protein	DOWN	0.33	0.06
DET1107 hypothetical protein	DOWN	0.29	0.05
DET1146 hypothetical protein	DOWN	0.36	0.07
DET1186 hypothetical protein	UP	2.9	1.12
DET1206 metM homoserine dehydrogenase	DOWN	0.34	0.09
DET1224 cobA 3 cob(I)alamin adenosyltransferase	DOWN	0.43	0.04
DET1278 nusB N utilization substance protein B	DOWN	0.25	0.07
DET1284 hypothetical protein	DOWN	0.38	0.06
DET1285 serine protease, DegP/HtrA family	UP	2.9	0.58
DET1286 serine protease, DegP/HtrA family	UP	3.9	1.28
DET1377 hypothetical protein	UP	3.0	0.70
DET1379 auxin responsive GH3 protein homolog	UP	3.7	0.29
DET1419 transcriptional regulator, AbrB family	DOWN	0.29	0.15
DET1511 hypothetical protein	DOWN	0.14	0.12
DET1544 reductive dehalogenase anchoring protein	UP	3.5	0.59
DET1569 hypothetical protein	UP	3.0	0.65
DET1624 hypothetical protein	UP	2.6	0.39

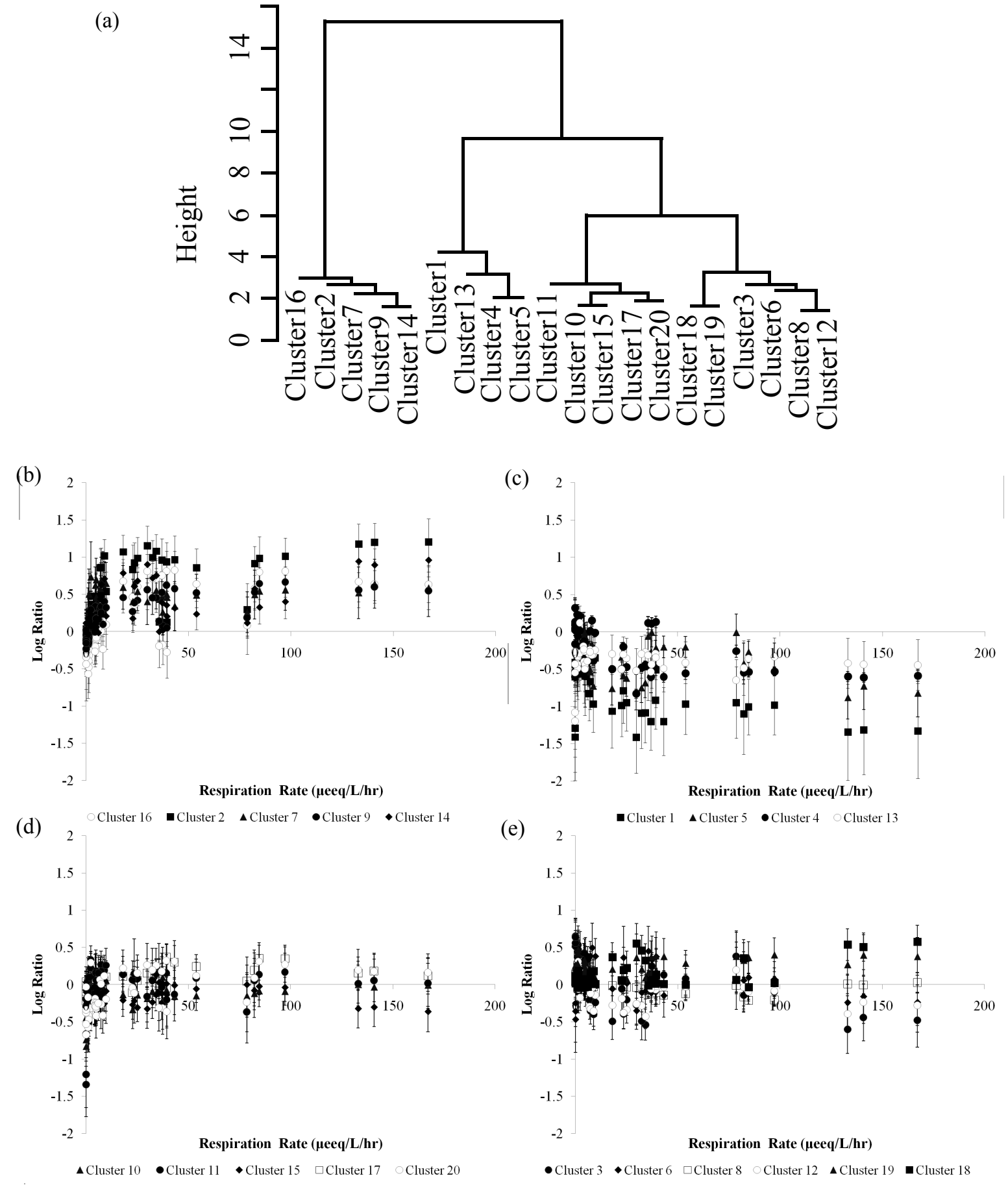
1 Table 1. The 43 elements that are similarly regulated amongst all four Batch vs. PSS samples
2 with the transcript name, regulation (up or down when comparing PSS to batch level), average
3 natural value ratio recorded, and the standard deviation.

	PCE1	PCE2	TCE1	TCE2
PCE1	1	0.72	0.78	0.78
PCE2	0.72	1	0.66	0.55
TCE1	0.78	0.66	1	0.84
TCE2	0.78	0.55	0.84	1

Figure 1. Comparison of the Batch Control and PSS fed cultures log ratio correlations. The correlogram displays the similarity of the experimental replicates TCE 1&2 and PCE 1&2 be correlating the log ratio for every gene transcript across experiments.

Experiment Title	Culture Title	Electron Acceptor	EA Feed Rate (μeeq/L/hr)	Electron Donor Source	ED:EA Ratio (eeq ratio)	Time (Days)	Respiration Rate (μeeq/L/hr)
PCE High	HiP1	PCE	258	Butyrate	3.4	1	140
	HiP2	PCE	234	Butyrate	3.8	1	130
	HiP3	PCE	282	Butyrate	3.1	1	170
PCE High Low	HLH1_INHIB	PCE	456	Butyrate	1.9	7	95
	HLH2_INHIB	PCE	504	Butyrate	1.7	7	110
	HLL1	PCE	7.2	Butyrate	4.1	7	6.3
	HLL2	PCE	7.2	Butyrate	4.1	7	6.2
	HLL3	PCE	7.2	Butyrate	3.3	7	6.7
PCE Half Butyrate	PHB1	PCE	102	Butyrate	0.73	7	85
	PHB2	PCE	45.6	Butyrate	1.7	7	39
	PHB3	PCE	108	Butyrate	0.72	7	97
PCE Lactate or Butyrate	PLL1	PCE	45	Lactate	2.1	7	43
	PLL2	PCE	38.4	Lactate	1.3	7	37
	PLB1	PCE	30	Butyrate	1.5	7	30
	PLB2	PCE	58.2	Butyrate	1.8	7	54
PCE Hydrogen	H2A1	-	-	H_2	-	1.5	-
	H2A2	-	-	H_2	-	1.5	-
	H2PB1	PCE	1.7	H_2	0.74	1.5	9.7
	H2PB2	PCE	1.5	H_2	0.85	1.5	7.3
PCE 0, YE, FYE	P0FY01	PCE	3	-	-	3	1.3
	P0FY02	PCE	3	-	-	3	1.3
	P0FYF1	PCE	4.8	FYE	3.2	7	4.1
	P0FYF2	PCE	5.4	FYE	2.5	7	4.9
	P0FYY1	PCE	4.8	YE	2.8	7	4.5
	P0FYY2	PCE	4.8	YE	2.8	7	4.3
PCE 3 Rates	P3A1	PCE	25.2	Butyrate	2.5	4	25
	P3A2	PCE	22.8	Butyrate	2.8	4	23
	P3B1	PCE	4.2	Butyrate	4.8	4	4.5
	P3B2	PCE	4.8	Butyrate	4.2	4	4.9
	P3C1	PCE	0.6	Butyrate	17	4	1
	P3C2	PCE	0.6	Butyrate	16	4	0.9
TCE 3 Rates	T3A1	TCE	34.4	Butyrate	3.8	4	34
	T3A2	TCE	23.6	Butyrate	3.2	2	23
	T3B1	TCE	6.8	Butyrate	5.1	4	6.9
	T3B2	TCE	7.2	Butyrate	4.8	4	7.3
	T3C1	TCE	1.6	Butyrate	11	4	1.5
	T3C2	TCE	1.4	Butyrate	12	4	1.4
DCE 3 Rates	D3A1	DCE	9	Butyrate	2.6	4	18
	D3A2	DCE	16	Butyrate	3.0	2	32
	D3B1	DCE	4.4	Butyrate	3.0	4	8.9
	D3B2	DCE	4.1	Butyrate	3.2	4	8.2
	D3C1	DCE	1.1	Butyrate	5.5	4	2.3
	D3C2	DCE	1.2	Butyrate	5.4	4	2.3
DCE 10	D1_INHIB	DCE	120	Butyrate	2.0	7	37
	D2_INHIB	DCE	114	Butyrate	1.8	7	35
	D3_INHIB	DCE	120	Butyrate	2.7	7	39
Chlorophenols	CpA1	DCP	0.31	Butyrate	9.2	3	N/D
	CpA2	DCP	0.31	Butyrate	9.9	3	0.36
	CpB1	DCP	0.56	Butyrate	6.8	3	0.42
	CpB2	DCP	0.56	Butyrate	6.8	3	0.42
Decay	DecayA1	-	-	-	-	7	-
	DecayB1	-	-	-	-	3	-
	DecayB2	-	-	-	-	3	-

Table 2. Experimental conditions for all the 12 investigations comparing the PSS versus a 3 day starved control. The experimental title provides a brief description of the overall experiment where the culture title designates the electron acceptor (PCE, TCE, DCE, DCP, or none (-)) and electron donor source (butyrate, lactate, hydrogen, yeast extract (YE), fermented yeast extract (FYE), hydrogen, or none (-)) type. Note, the experiments that received butyrate and lactate also were treated with YE.



1 Figure 3. Biological breakdown of the clusters. (a) A Euclidean distance and Ward cluster
2 dendogram comparing the similarities of the clusters. (b-e) Scatterplots plotting the centroid value
3 with 95% confidence intervals about the mean against the experimental respiration rates for all
4 chlorinated ethenes.

RDase	Anchor	Predicted Histidine Kinase Sensory Box	Predicted DNA Binding Response Reg.	Predicted MarR Regulator	Annotated Lipo-protein	Other Annotations (Including Hypothetical)		
DET0079 - *tceA*	DET0078							
	0.97							
DET0318 - *pceA*	DET0319	DET0315	DET0316		DET0321	DET0317	DET0320	
	0.94	0.18	0.30		-0.10	-0.12	0.69	
DET1559	DET1558	DET1560	DET1561		DET1557	DET1556		
	0.69	0.60	0.70		0.61	0.78		
DET0173	DET0175	DET0171	DET0170		DET0174	DET0172	DET0176	
	0.95	-0.25	-0.19		0.94	0.39	0.58	
DET0180	DET0181	DET0178	DET0177			DET0179		
	0.68	0.54	0.48			0.34		
DET1535	DET1534			DET1536		DET1533	DET1532	
	0.94			-0.63		0.41	0.49	
DET1538	DET1537	DET1539	DET1540					
	0.68	0.48	-0.10					
DET1545	DET1544					DET1541	DET1542	DET1543
	0.77					0.33	0.10	-0.31

Table 3. Correlation scores of RDases that meet the background cutoff to neighboring annotated genes including putative response regulators. The solid black line separates the RDases based on their clustering and response to respiration rate (RDases responding positively to respiration rate above the black line, negatively below) displayed in Figure 4. A negative correlation score indicates an inverse correlation. Values >0.25 or <-.25 are considered significant by the one-tailed t-test with N=49.

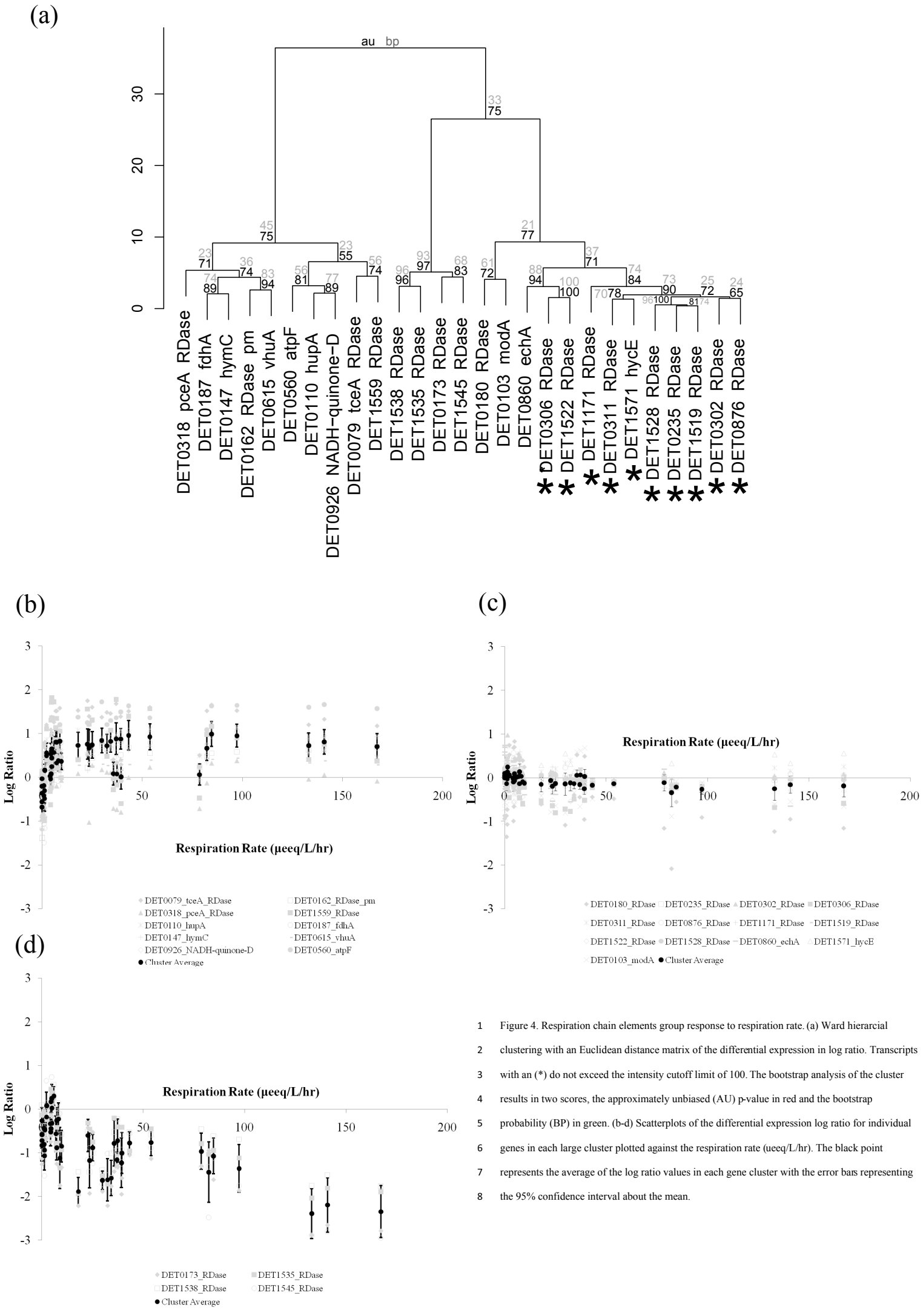


Figure 4. Respiration chain elements group response to respiration rate. (a) Ward hierarcial clustering with an Euclidean distance matrix of the differential expression in log ratio. Transcripts with an (*) do not exceed the intensity cutoff limit of 100. The bootstrap analysis of the cluster results in two scores, the approximately unbiased (AU) p-value in red and the bootstrap probability (BP) in green. (b-d) Scatterplots of the differential expression log ratio for individual genes in each large cluster plotted against the respiration rate (ueeq/L/hr). The black point represents the average of the log ratio values in each gene cluster with the error bars representing the 95% confidence interval about the mean.

mRNA ID	Correlation Score	mRNA ID	Correlation Score
DET0320 hypothetical protein	0.90	DET1407 BNR/Asp box repeat domain protein	0.98
DET1559 RDase	0.78	DET0078 tceA anchoring protein	0.97
DET0170 DNA binding response regulator	0.76	DET0511 hypothetical protein	0.93
DET1543 hypothetical protein	0.69	DET0112 [Ni/Fe] hydrogenase	0.93
DET0171 sensory box sensor histidine kinase	0.68	DET1600 twin arginine translocation TatB	0.93
DET0177 DNA binding response regulator	0.67	DET0117 transcriptional regulator	0.92
DET1556 hypothetical protein	0.67	DET0352 hypothetical protein	0.91
DET0319 RDase anchoring protein	0.67	DET0191 iron sulfur cluster binding protein	0.91
DET1557 lipoprotein, putative	0.63	DET0320 hypothetical protein	0.90
DET0318 RDase	0.62	DET0145 [Fe] hydrogenase, HymA	0.89
DET1560 sensory box sensor histidine kinase	0.61	DET0111 [Ni/Fe] hydrogenase	0.89
DET0178 sensor histidine kinase	0.60	DET0187 formate dehydrogenase	0.89
DET1561 DNA binding regulator, LuxR family	0.55	DET0186 formate dehydrogenase	0.88
DET0316 DNA binding response regulator	0.51	DET0343 ftsZ-1 cell division protein	0.87
DET0305 sensory box sensor histidine kinase	0.48	DET0996 rpmG ribosomal protein L33	0.87
DET1540 DNA binding response regulator	0.40	DET1225 hypothetical protein	0.87
DET0315 sensory box sensor histidine kinase	0.33	DET0997 tuf translation elongation factor Tu	0.87
DET0322 hypothetical protein	0.33	DET0994 nusG transcription antitermination	0.87
DET1558 RDase anchoring protein	0.29	DET1417 purH cyclohydrolase	0.86
DET1164 iron sulfur cluster binding protein	0.25	DET0001 dnaA replication initiator	0.86

1 Table 4. Correlation scores of *tceA* to RDases and response regulators adjacent to RDases and

2 correlation scores of *tceA* to all transcripts on DET s genome represented on the microarray.

3

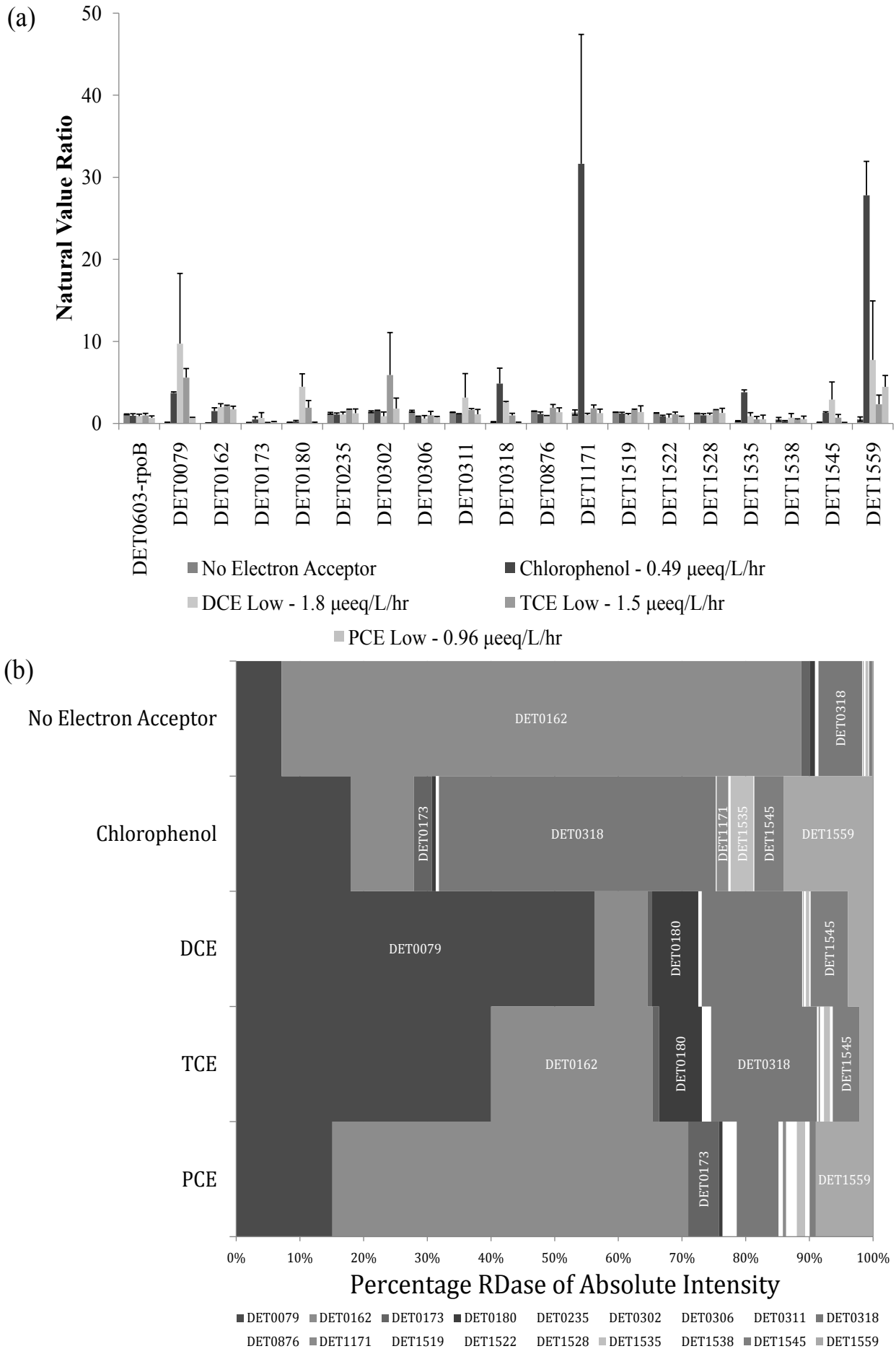
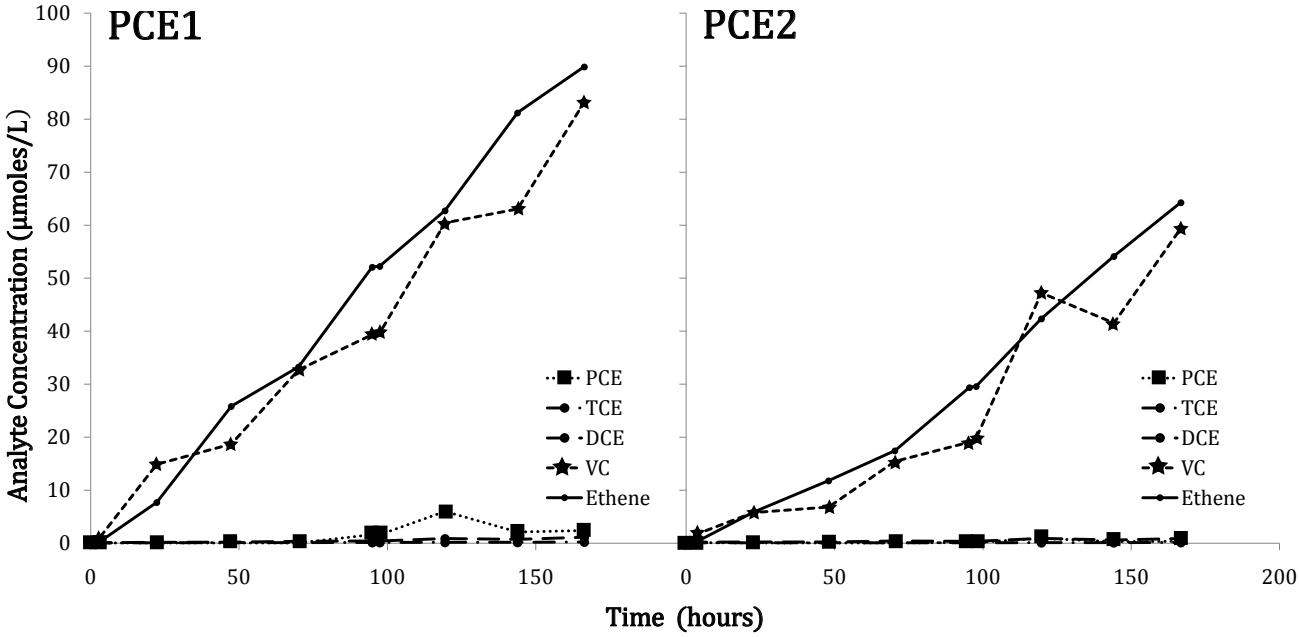
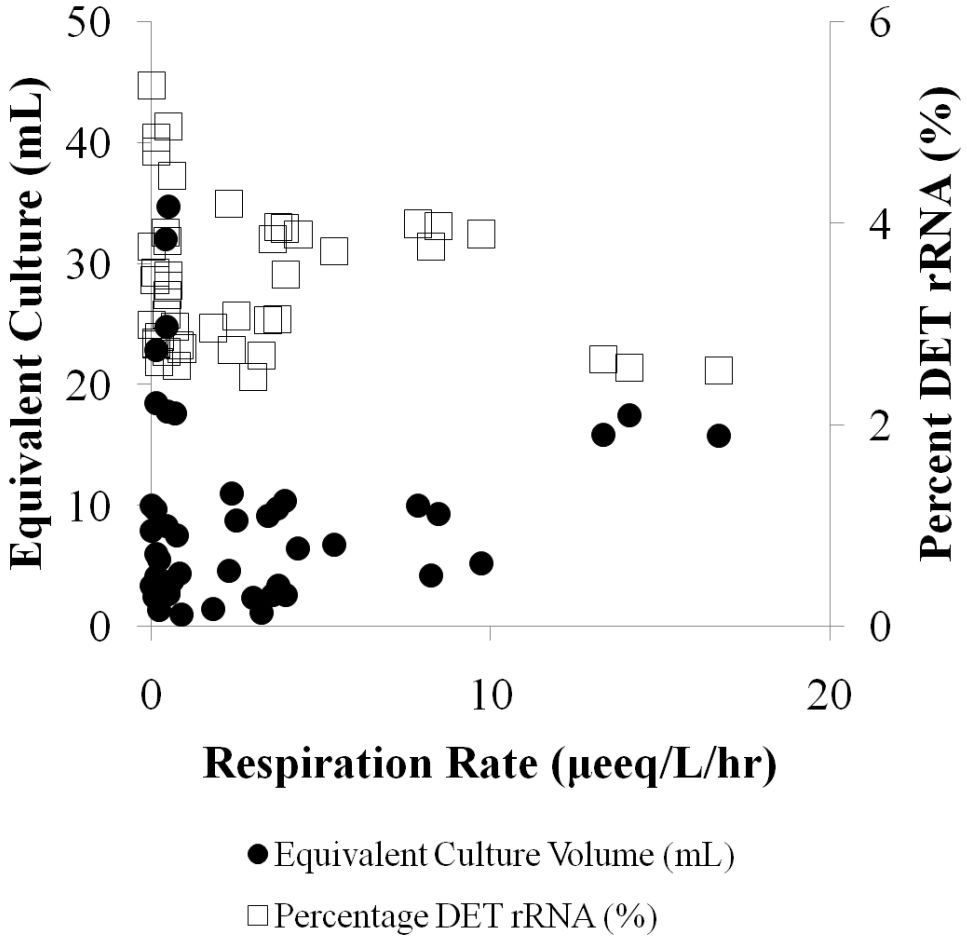


Figure 5. RDase distribution across different electron acceptors with the (a) natural value ratio compared to a 3-day starved culture for the 17 RDase and the *rpoB* transcript is plotted for the PCE, TCE, DCE, DCP, and No Acceptor fed conditions. Error bars represent standard error across biological replicates. (b) The absolute processed intensity value distribution of the RDases for the No Acceptor, DCP, DCE, TCE, and PCE cultures respectively moving inwards from the outer ring of the pie chart.

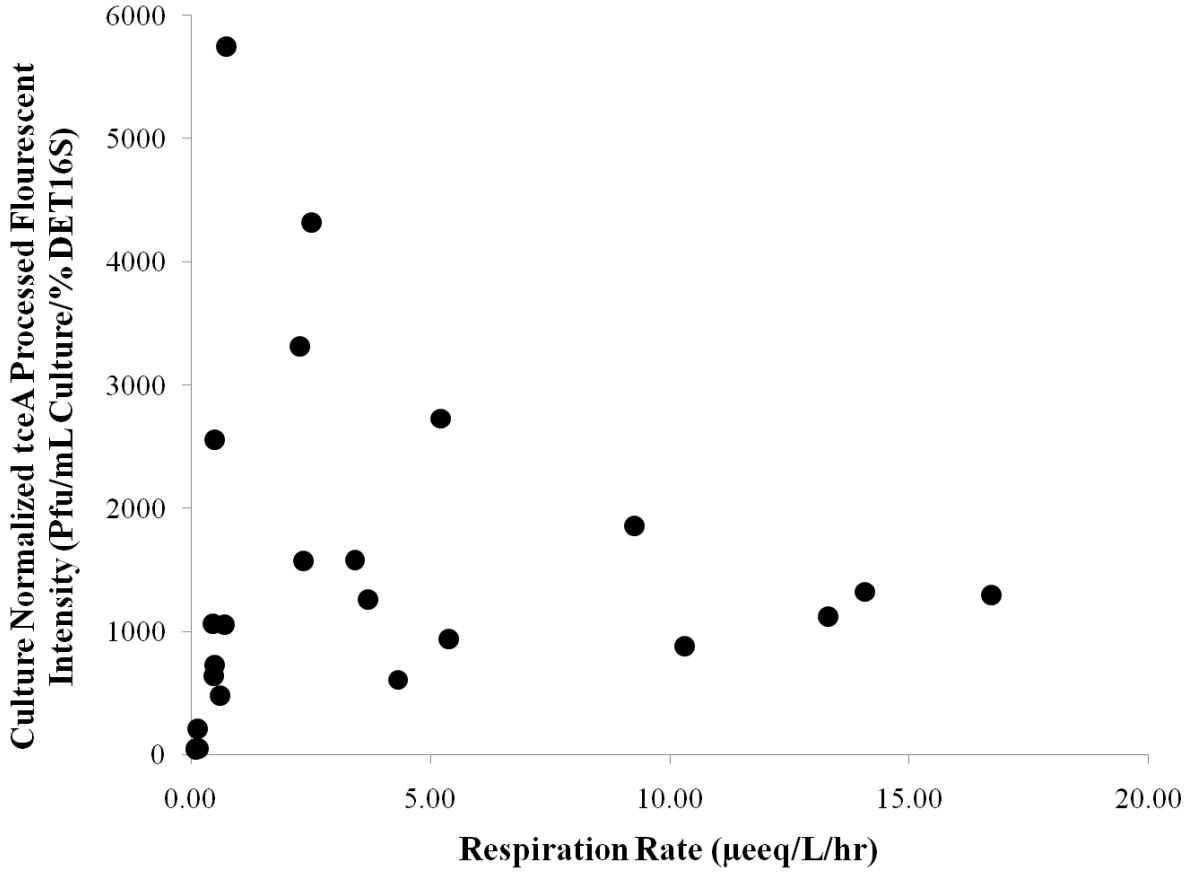

PCE1
PCE2
Analyte Concentration (µmoles/L)
Time (hours)
PCE
TCE
DCE
VC
Ethene
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200

Transcript ID and Description	Regulation	Ratio	Transcript ID and Description	Regulation	Ratio	Transcript ID and Description	Regulation	Ratio
DET0014 hypothetical protein	UP	2.7	DET0754 hypothetical protein	UP	2.7	DET1146 hypothetical protein	DOWN	0.36
DET0035 gmk guanylate kinase	DOWN	0.42	DET0755 hypothetical protein	UP	3.8	DET1173 fwdE family protein	UP	2.6
DET0036 hypothetical protein	DOWN	0.44	DET0861 hydrogenase, EchB subunit	DOWN	0.37	DET1186 hypothetical protein	UP	2.9
DET0108 endonuclease/exonuclease/phosphatase	UP	2.2	DET0862 hydrogenase, group 4, EchC subunit	DOWN	0.35	DET1206 metM homoserine dehydrogenase	DOWN	0.34
DET0137 mgsA methylglyoxal synthase	DOWN	0.31	DET0864 hydrogenase subunit HymB	DOWN	0.37	DET1224 cobA 3 cob(I)alamin adenosyltransferase	DOWN	0.43
DET0180 reductive dehalogenase	UP	5.5	DET0908 arsenical pump membrane protein	UP	7.8	DET1278 nusB N utilization substance protein B	DOWN	0.25
DET0181 reductive dehalogenase anchoring protein	UP	5.1	DET0909 hypothetical protein	UP	2.8	DET1284 hypothetical protein	DOWN	0.38
DET0297 hypothetical protein	UP	4.9	DET0945 ABC transporter, permease protein	DOWN	0.43	DET1285 serine protease, DegP/HtrA family	UP	2.9
DET0298 hypothetical protein	UP	4.5	DET0964 recJ single stranded DNA exonuclease RecJ	DOWN	0.38	DET1286 serine protease, DegP/HtrA family	UP	3.9
DET0322 hypothetical protein	DOWN	0.35	DET1021 hypothetical protein	DOWN	0.20	DET1300 universal stress protein family	DOWN	0.27
DET0368 proS prolyl tRNA synthetase	UP	2.7	DET1031 hypothetical protein	DOWN	0.40	DET1377 hypothetical protein	UP	3.0
DET0420 hypothetical protein	DOWN	0.25	DET1035 tryptophan synthase subunit beta	UP	3.2	DET1379 auxin responsive GH3 protein homolog	UP	3.7
DET0423 hypothetical protein	UP	4.2	DET1038 ileS isoleucyl tRNA synthetase	UP	2.5	DET1419 transcriptional regulator, AbrB family	DOWN	0.29
DET0485 rplX ribosomal protein L24	DOWN	0.43	DET1051 hypothetical protein	UP	3.8	DET1448 hypothetical protein	UP	2.8
DET0558 atpB ATP synthase F0, A subunit	DOWN	0.21	DET1057 hypothetical protein	UP	2.3	DET1511 hypothetical protein	DOWN	0.14
DET0560 atpF ATP synthase F0, B subunit	DOWN	0.33	DET1063 DNA binding regulator, LuxR family	UP	2.7	DET1544 reductive dehalogenase anchoring protein	UP	3.5
DET0561 atpH ATP synthase F1, delta subunit	DOWN	0.33	DET1068 recombinase, phage integrase family	DOWN	0.30	DET1545 reductive dehalogenase	UP	3.3
DET0562 atpA ATP synthase subunit A	DOWN	0.41	DET1092 DNA methylase	DOWN	0.41	DET1569 hypothetical protein	UP	3.0
DET0673 hypothetical protein	UP	3.6	DET1105 hypothetical protein	DOWN	0.27	DET1624 hypothetical protein	UP	2.6
DET0721 hypothetical protein	DOWN	0.36	DET1106 hypothetical protein	DOWN	0.33			
DET0736 oxidoreductase, dehydrogenase/reductase	UP	2.7	DET1107 hypothetical protein	DOWN	0.29			

(a)


Equivalent Culture (mL)
Percent DET rRNA (%)
Respiration Rate (µeeq/L/hr)
Equivalent Culture Volume (mL)
Percentage DET rRNA (%)

(b)


Culture Normalized tceA Processed Flourescent Intensity (Pfu/mL Culture/% DET16S)
Respiration Rate (µeeq/L/hr)